REMARKS

Claims 1-9, 11-17 and 59-64 presently appear in this case. No claims have been allowed. The official action of June 27, 2006, has now been carefully studied. Reconsideration and allowance are hereby respectfully urged.

Briefly, the present invention relates to a method of altering gene expression in a population of human embryonic stem cells by introducing into the population of human embryonic stem cells a polynucleotide that is operably linked to a promoter and contains a gene expression altering sequence. It is possible to obtain a transfection efficiency greater than that obtainable by means of electroporation by use of an appropriate transfection reagent.

Claims 1-9, 11-17 and 59 have been rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The examiner states that this is in the nature of a new matter rejection. The examiner considers that the amendment to the claims introduces new matter into the disclosure with regard to the recitation that the method of altering gene expression requires a transfection efficiency greater than obtainable by means of electroporation. The examiner states that applicants have not pointed specifically to where support for the amendment to the

claims can be found. This rejection is respectfully traversed.

Claims 1 and 11 have now been amended to clarify that the transfection reagent used is one that provides a transfection efficiency greater than that obtainable by electroporation. MPEP ¶2163, which sets forth the guidelines for examination of claims under the 35 U.S.C. 112, first paragraph, written description requirement, states in section II A.3(b), second paragraph:

> To comply with the written description requirement of 35 U.S.C. 112, para. 1, … each claim limitation must be expressly, implicitly, or inherently supported in the originally filed disclosure.

Note that MPEP ¶2163.02, states:

> The subject matter of the claim need not be described literally (i.e., using the same terms or *in haec verba*) in order for the disclosure to satisfy the description requirement.

Here, the specification as filed stated that, in its broadest aspect, the present invention included transfecting the cells in any manner, such as by means of a cationic polymer transfection reagent or by electroporation; see, for example, paragraph [0013] and paragraph [0015]. However, in paragraph [0043], the present specification clearly states that, while electroporation was found to be the method of choice for introducing foreign DNA into murine ES cells, the

present inventors had found that "although human ES cells can be transfected by electoporation, improved results were obtained by transfection in the presence of cationic polymers." Thus, it is clear that the present inventors were in possession of the concept of using a transfection reagent, such as cationic polymers, in order to obtain improved results over that which can be obtained by electroporation when transfecting human ES cells.

It is true that ExGen 500 was the only one shown to have a transfection efficiency substantially greater than electroporation, and indeed three other transfection reagents were found to have a transfection efficiency not substantially greater than that obtained by electroporation. Nevertheless, the concept of the use of transfection reagents that provide efficiency better than that of electroporation is present in the specification; see paragraph [0045]. Thus, specifying in the claims that the transfection reagent used is one that provides a transfection efficiency greater than that of electroporation, is not new matter. While the present specification provides only a single example of a transfection agent whose efficience is greater than electroporation, those of ordinary skill in the art reading the present specification would understand that the present inventors did not consider the present invention to be limited to ExGen 500. Rather,

those of ordinary skill in the art reading the present specification would understand that experiments with other transfection reagents, known in the art, could be conducted in the same manner as disclosed in Example 1, in order to find other transfection reagents that work in a manner similar to that shown for ExGen 500 to provide an increased efficiency over electroporation. Indeed, subsequent to the filing of the present application, other transfection reagents having an efficiency greater than that of electroporation have been found without using undue experimentation. Note, for example, the attached publication of Darr, et al., "Overexpression of *NANOG* in Human ES Cells Enables Feeder-Free Growth While Inducing Primitive Ectoderm Features," *Development*, 133:1193-1201 (2006). In this publication, transfection was obtained with calcium phosphate.

Accordingly, the expression in question does not represent new matter as it was at least implicitly present in the specification and those of ordinary skill in the art reading the present specification as a whole would have understood that the inventors had considered this to be part of their invention. Reconsideration and withdrawal of this rejection are therefore respectfully urged.

In any event, it should be noted that new claims 62-64 do not have the language that the examiner finds

objectionable and so this rejection is inapplicable thereto. These claims are not subject to the previously appearing prior art rejections as they require that the polynucleotide not contain viral genes. This feature is sufficient to define over the prior art previously applied for the reasons advanced in applicants' previous response.

Claims 1-3, 7-9, 11-13, 16, 17 and 59 have been rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The examiner states that, while the specification provides adequate guidance with regard to transfection of hES cells in the presence of a cationic non-lipid polymer reagent with a polynucleotide operably linked to a promoter, wherein the polynucleotide encodes a fluorescent protein or antibiotic resistant protein, the specification fails to describe any other species within the genus of gene expression altering sequences that show measurably different gene expression after introduction of the polynucleotide while retaining the pluripotent character of the cells as instantly claimed and encompassed by the claims with particularity to indicate that applicants had possession of the claimed invention. The examiner states that the specification does not provide guidance as to which exogenous nucleic acids, enhancers, promoters, or transcription activators could be used to

produce the required effects, namely, measurably different gene expression before and after transfection, while maintaining the pluripotent character of the hES cells. This rejection is respectfully traversed.

The advance of the presently claimed invention that defines over the prior art, is the discovery that, while human cells cannot be transected with reasonable efficiency using the same technique that is commonly and successfully used for transfecting mouse ES cells with good efficiency, i.e., electroporation, human ES cells can be transfected with excellent efficiency using certain transfection reagents, such as ExGen 500. The present inventors do not claimed to have made any new advances with respect to the exogenous nucleic acids, enhancers, promoters, or transcription activators that can be transfected into the ES cells using the present invention.

The examiner states that the present specification only discloses a polynucleotide encoding a fluorescent protein or an antibiotic resistant protein. However, these experiments establish the proof-of concept that some transfection reagents, as opposed to electroporation, can be used to obtain excellent efficiency when transfecting hES cells. In general, the art of transfecting cells with a polynucleotide such that the gene expression prior to

introducing the polynucleotide is measurably different from gene expression after introducing the polynucleotide is well established and was well within the skill of one of ordinary skill in the art at the time that the present invention was made. The proof-of-concept in the examples of the present specification establishes that, not only can this be done at an efficiency greater than that obtainable by electroporation, but that the pluripotent character of the cells is retained. In view of this proof-of-concept using polynucleotides encoding antibiotic resistant proteins or fluorescent proteins, there is no reason to believe that any other polynucleotide might not be transfected in the same manner and achieve the same results as would be expected by one of ordinary skill in the art, while retaining the pluripotent character of the cells. It is clear that applicants considered this to be part of the present invention, particularly in light, for example, of the broad definition of "expression altering sequence" in paragraph [0038]. As applicants have not made any novel advances in such sequences but the invention is based on the insertion of such known sequences into human embryonic stem cells in a particular manner, it is apparent that applicant was in possession of the full scope of the generic invention. Reconsideration and withdrawal of this rejection is therefore respectfully urged.

Claims 1-9, 11-17 and 59 have been rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method for transfecting human ES cells by introducing a polynucleotide that does not contain viral genes into a population of human ES cells by transfection in the presence of a cationic non-lipid polymer reagent, wherein the polynucleotide is operably linked to a promoter that encodes a fluorescent protein or an antibiotic resistant protein, the specification does not reasonably provide enablement for the breadth of the claims as claimed. The examiner states that the breadth of the claims encompassed using any of the transfection reagents and the introduction of any gene expression altering sequence into the hES cells so that introduction of the polynucleotide is measurably different from gene expression before and after introduction of the polynucleotide is not supported by an enabling disclosure. The examiner states that the Zwaka reference suggests that there is unpredictability in successful corporation of a gene in an hES cell by homologous recombination. This rejection is respectfully traversed.

The burden is on the examiner to show that it would take undue experimentation to practice the full scope of the invention claimed. As discussed above, one of ordinary skill in the art would expect that any kind of construct could be

transfected into the hES cells using the method of the present invention in view of the proof-of-concept that is present in the specification. Indeed, the laboratory of the present inventrs has published many papers showing the successful use of this process with many different types of constructs. Among these are the following, copies of which are attached hereto:

Darr, et al., "Overexpression of *NANOG* in Human ES Cells Enables Feeder-Free Growth While Inducing Primitive Ectoderm Features," *Development,* 133:1193-1201 (2006). In this paper, the construct is human NANOG-IRES-Puroycin.

Lavon, et al., "The Effect of Overexpression of Pdx1 and Foxa2 on the Differentiation of Human Embryonic Stem Cells into Pancreatic Cells," *Stem Cells*, 24:1923-1930 (2006). In this paper, the constructs are INS-EGFP, PDX1-EGFP, Actin-PDX1, and PGK-FOXA2.

Lavon, et al., "Differentiation and Isolation of Hepatic-Like Cells from Human Embryonic Stem Cells," *Differentiation*, 72:230-238 (2004). In this paper, the construct is ALB-EGFP.

Urbach, et al., "Modeling for Lesch-Nyhan Disease by Gene Targeting in Human Embryonic Stem Cells," *Stem Cells*, 22:635-641 (2004). In this paper, the construct is PHPR-Thyg.

Note further that this construct was successfully used for the purpose of homologous recombination.

Dhara, et al., "Gene Trap as a Tool for Genome Annotation and Analysis of X Chromosome Inactivation in Human Embryonic Stem Cells," *Nucleic Acids Research*, 32:3995-4002 (2004). In this paper, the construct is RET-gene trapC1010 vector.

Schuldiner, et al., "Selective Ablation of Human Embryonic Stem Cells Expressing a 'Suicide' Gene," *Stem Cells*, 21:257-265 (2003). In this paper, the constructs are pgk-neo, pgk-HSV-tk, and pgk-EGFP.

Eiges, et al., "Establishment of Human Embryonic Stem Cell-Transfected Clones Carrying a Marker for Undifferentiated Cells," *Current Biology*, 11:514-518 (2001). In this paper, the constructs are Rex-EGFP, pgk-EGFP, and CMV-EGFP.

It can thus be seen that, despite the examiner's comments, many different constructs have been used, without undue experimentation, using the process of the present invention. This confirms the fact that the proof-of-concept in the present specification would lead one of ordinary skill in the art to understand that the full scope of polynucleotides as are well known for the transfection of

other cell types, can be used in the present invention, and the later experimentation confirms this fact.

As to the examiner's comments about homologous recombination, note that the Urbach 2004 reference shows successful use of homologous recombination using the present invention.

Notwithstanding the above, it is urged that claims 3, 60, 61, 63 and 64 are allowable in their own right as they do not encompass homologous recombination.

Accordingly, reconsideration and withdrawal of this rejection is also respectfully urged.

Claims 1-9, 11-17 and 59 have been rejected under 35 U.S.C. 112, second paragraph, as being indefinite in the term "with a transfection efficiency greater than that obtainable by means of electroporation." The examiner states that this is a relative term, which renders the claim indefinite. The examiner states that the transfection efficiency of any given experiment is relative. This part of the rejection is respectfully traversed.

It is not understood why the examiner considers this term to be relative. The expression "A is greater than B" is not a relative expression. It clearly defines a condition. Example 1 shows how transfection efficiency can be compared

with electroporation. Thus, it can readily be determined, using such an experiment, whether any given transfection reagent permits a transfection efficiency greater than that obtainable by electroporation. The "requisite degree" is "greater than." This is not relative but fixed. Reconsideration and withdrawal of this part of the rejection is therefore respectfully urged.

The examiner further states that claim 1 recites the limitation "the nucleic acid" in the second to last line of the claim, but there is no antecedent basis for this term.

Claim 1 has now been amended to change the term "nucleic acid" to read "polynucleotide," which has antecedent basis in the claim.

The examiner states that claim 11 recites the limitation "nucleic acid," but that there is insufficient antecedent basis therefore.

Claim 11 has now been amended also to correct this objection. The term "nucleic acid" has now been amended to read "DNA sequence," which is supported earlier in the claim.

Accordingly, reconsideration and withdrawal of this entire rejection is respectfully urged.

It is submitted that all the claims now present in the case clearly define over the references of record and

fully comply with 35 U.S.C. 112. Reconsideration and allowance are therefore earnestly solicited.

Respectfully submitted,

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